AD_____

Award Number: W81XWH-05-1-0518

TITLE: MRI Study of Uninvolved Breast Tissue for Patients with Locally Advanced Breast Cancer Undergoing Pre-Operative Chemotherapy

PRINCIPAL INVESTIGATOR: Catherine Klifa, Ph.D.

CONTRACTING ORGANIZATION: University of California
San Francisco, California, 94143

REPORT DATE: August 2006

TYPE OF REPORT: Revised Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01-08-2006	Revised Final	1 AUG 2005 - 31 JUL 2006

4. TITLE AND SUBTITLE

MRI Study of Uninvolved Breast Tissue for Patients with Locally Advanced Breast Cancer Undergoing Pre-Operative Chemotherapy

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-05-1-0518

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Catherine Klifa, Ph.D.

E-Mail: catherine.klifa@radiology.ucsf.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of California
San Francisco, California, 94143

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Purpose: To study possible associations between the MRI morphological patterns of the primary breast tumor and properties of the surrounding host tissue. Scope: Identification of such properties of host tissue may be useful in characterizing breast tissue for earlier stage breast cancers. Major findings: We have developed MRI algorithms necessary to measure signal enhancement ratios in non-tumor tissue using the existing database of patients who underwent neoadjuvant chemotherapy. Results: We found that change in uninvolved breast tissue volume with treatment was correlated with tumor phenotypes and was shown to be predictive of recurrence. Significance: Host tissue changes with treatment can potentially provide information about pre-cancerous tissue that may have applicability to risk assessment.

15. SUBJECT TERMS
Breast Cancer Research, treatment response, chemotherapy treatment, normal breast tissue study, Magnetic Resonance Imaging, quantitative measures of breast tissue changes with treatment. Magnetic Resonance Imaging, Quantitative measures of breast tissue changes with treatment.

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
					USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
U	U	U	UU	37	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

Introduction:

The purpose of this retrospective study was to better understand the relationship between breast cancer tumors and their normal "host" tissue, by studying the MRI characteristics of normal tissue in patients with invasive cancers. Since there is a strong association between the tumor morphologic patterns on MRI with both treatment outcome and survival, we hypothesized that the imaging phenotype reflects tumor growth patterns that are influenced by the normal host tissue. We wanted to study possible associations between the morphological patterns of the primary breast tumor and properties of the surrounding host tissue. Our goal was to verify whether a change in normal breast tissue with treatment had any implications on the outcome (recurrence).

Body:

MRI is a very effective imaging method for staging the extent of cancer in the breast [1,2]. Our group and others are studying MRI's effectiveness for assessing tumor response to pre-operative chemotherapy for women with locally-advanced breast cancer [3-5].

Sixty-eight women were previously enrolled in a breast MRI study at our institution and underwent MRI exams prior to the start of neoadjuvant adriamycin/cytoxan (AC) chemotherapy (MRI1), following one cycle of AC (MRI2) and at the completion of chemotherapy, prior to surgery (MRI3). The MRI exam included a dynamic contrast-enhanced 3D sagittal T1-weighted sequence, and a diffusion-weighted single shot fast-spin echo sequence (see MR parameters in table 1). As defined in Task 1 of the Statement of Work, we reviewed our neoadjuvant MRI database to select cases with initial disease extended over less than 2 quadrants. After review, this retrospective study included 42 neoadjuvant patients (mean age 48.56 years (range 29.7 - 71.5)) Median follow-up was 178 weeks. Pathology and radiology reports were available for all patients. Total follow-up was 3 years.

MR Protocol (1.5T GE)	MR Parameters
T1-weighted 3D fast gradient echo 3DFGRE	TR/TE=8.4/4.2ms, NEX2, 256x256 matrix, FOV 20cm, Slice Thickness 2mm, no gap between slices
Bilateral Axial diffusion-weighted	TR/TE=6.2/6.1ms, b=600, FOV 35cm, Slice Thickness 5mm

***Table 1**: MRI parameters*

In order to study breast tissue around the tumor region, we applied several modifications to image analysis routines. These modifications allowed to quantify MRI background tissue (or "normal tissue") enhancement as well as enhancement values at different locations on and around tumors. Magnetic Resonance Signal Enhancement Ratio (SER) values have been histopathologically shown to positively correlate with microvessel density within tumor [5]. A complete description of the SER measure applied to tumor regions is provided in the appendix, on page 6 and figure 1. However SER in non-enhancing tissue has yet to be characterized: characteristics of radiographically normal breast tissue such as microvessel density may be associated with progression of disease.

In this project we lowered the enhancement threshold necessary to detect MRI tumor-enhancing regions, to insure that slow or non-enhancing tissue regions could be analyzed. Figure 1 shows different SER maps obtained of the same region using a "tumor" enhancement threshold (70%) shown in 1(b) and a "normal tissue" enhancement threshold (20%) in 1(c).

In order to study the enhancement of regions around tumors, we extracted regions of interest in non-enhancing breast tissue located close to the tumor and quantified SER values in these regions at each exam. In figure 2 of the appendix we show the various regions used to measure SER values around the tumor. The empirical 20% enhancement threshold in this project allowed to record normal tissue slower enhancement while still providing complete information about the tumor.

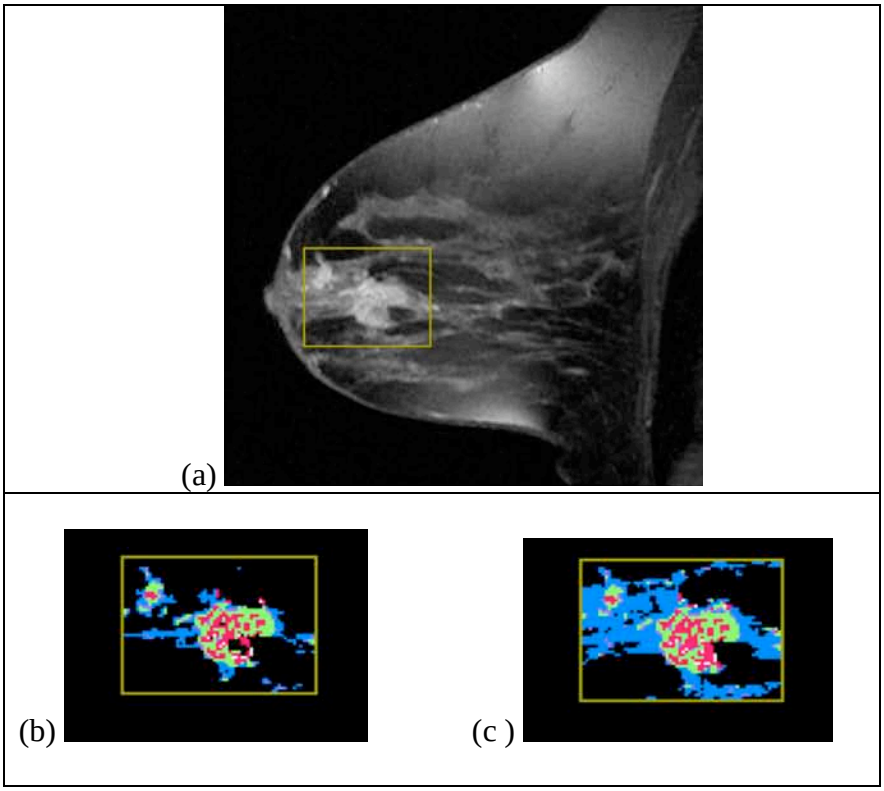


Figure 1: *(a) Sagittal original contrast-enhanced T1-weighted MR image presenting the rectangular region including tumor (bright values) and breast tissue (darker gray values) and fat (black); (b) corresponding SER map obtained using 70% enhancement threshold to extract tumor region (red and green); (c) corresponding SER map using 20% enhancement threshold to measure enhancement in normal tissue (blue) and lesion (red and green).*

For all patients, tumor morphologic patterns, or "imaging phenotypes" [5] were categorized according to the degree of tumor containment from 1, denoting well-circumscribed uni-centric masses to 5, corresponding to infiltrative tumors with ill-defined borders. Figure 2 presents the Maximum Intensity Projection ("MIP") created from contrast-enhanced MR data showing examples of the various phenotypes 1 to 5.

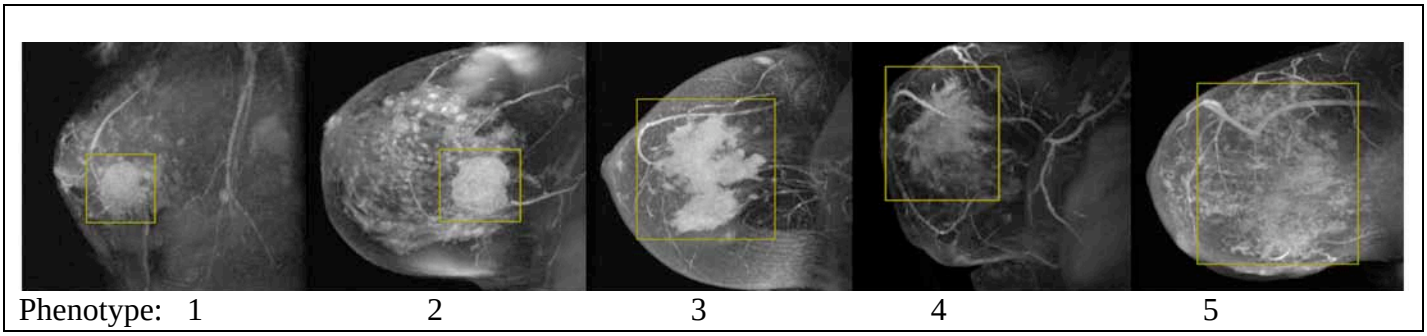


Figure 2*: Imaging phenotypes used in this project: 1 (well-circumscribed uni-centric mass) to 5 (infiltrating tumors with ill-defined borders).*

Tumor volumes were quantified from initial enhancement dynamic data on both MRI1 (baseline) and MRI3 (before surgery) [3]. As described in the appendix (see appendix pages 6-7 and figure 1), tumor volumes were defined using the SER technique, which has been developed and tested by our group.
In order to quantify the uninvolved breast tissue volume, we used our semi-automated technique [6] that performs the segmentation of breast tissue from fat regions without user interaction, therefore avoiding threshold decision issues. This technique is particularly useful to correct for partial voluming issues (voxels containing both fatty and fibroglandular tissue, and therefore hard to classify into one or the other

category), inherent to MR data reconstruction. Figure 3 presents our semi-automated segmentation technique to extract breast tissue volume from pre-contrast MR data.

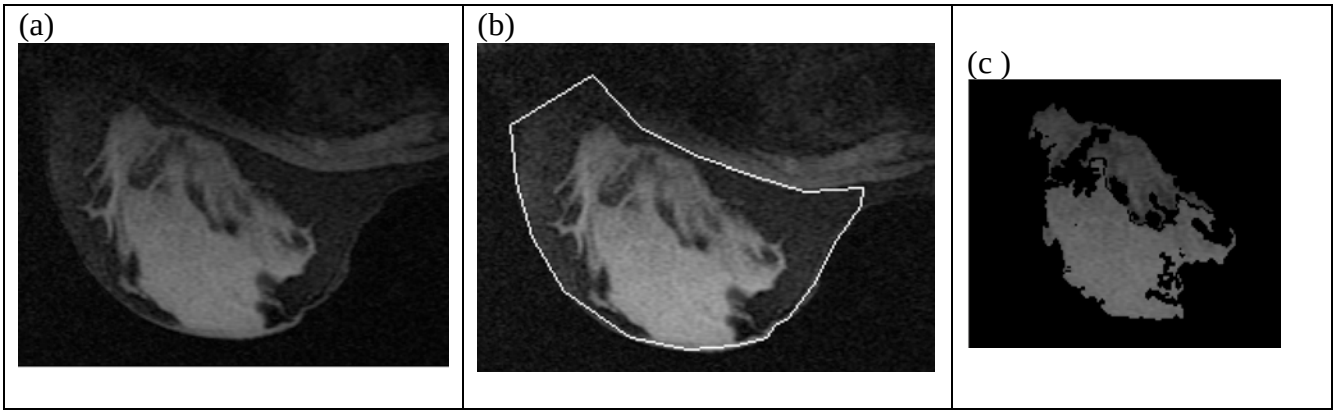


Figure 3: *(a) original axial pre-contrast T1 weighted MR data; (b) Delineation of global breast volume (performed by user) and (c) Result of the automated segmentation of breast tissue from fat. The resulting image presents gray level variations related to partial voluming present in the original data.*

We used this technique to measure the total fibroglandular volume on pre-contrast images.
Since the total fibroglandular volume on pre-contrast MR includes the tumor region, in order to obtain the final volume of **non-affected tissue only**, we subtracted the tumor volume obtained through the SER map from the total fibroglandular volume.
Table 2 summarizes the various measures performed on the MR data in this project.

Measures	Location of measure	Extracted from
Mean SER	Tumor	SER map
Mean SER	Normal tissue around tumor	SER map
Total breast tissue volume	Whole breast	Pre-contrast T1-weighted
Tumor volume	Tumor	SER map
Non-tumor breast tissue volume	Normal tissue around tumor	Total breast volume – tumor volume

Table 2*: Summary of various MR measures*

Statistical analyses investigated correlations between primary tumor characteristics and host tissue measurements. We measured the differences in mean SER on MR1, MR2 and MR3 for all patients, for both tumor and surrounding regions. We studied the correlation between change in uninvolved breast tissue volume between the first, second and last MRI of each patient, with change in tumor volume. We also studied the correlation between change in uninvolved breast tissue volume and recurrence. Statistical analysis including Spearman Rho multivariate correlation was used to investigate the association between all measurements made in the host tissue and tumor characteristics.

Results:

Ten patients recurred. We found that non-tumor mean SER did not change significantly with one cycle of chemotherapy, however the value of non-tumor mean SER after 1 cycle of chemotherapy was an independent predictor of disease free survival. These results suggest that we may use non-tumor SER to risk stratify patients receiving neoadjuvant chemotherapy. These preliminary results were presented at the American Society for Therapeutic Radiology and Oncology (ASTRO) in November 2006. We also submitted a manuscript to the American Journal of Roentgenology. The appendix of this report presents this submitted manuscript.

We also confirmed the findings from previous studies in which we had observed a strong association between the initial (pre-treatment) tumor morphologic patterns on MRI with treatment outcome [1]. We

found that change in non-tumor breast tissue volume after chemotherapy does relate to tumor phenotype ($p<0.037$), and breast tissue volume before treatment ($p<0.029$). Change in non-tumor breast tissue volume after chemotherapy was also predictive of recurrence ($p<0.06$). However we found that change in uninvolved breast tissue volume with treatment did not relate to change in tumor volume.

Key Research Accomplishments

- Defined new enhancement threshold to quantify non-tumor low-enhancing tissue on MRI data
- Defined new semi-automated technique to segment breast tissue around tumor, and automatically define various locations around tumor
- Measured differences in MRI measures between scans acquired several times during treatment
- Found that change in non-tumor volume with treatment was predictive of reccurrence
- Found that non-tumor mean Signal Enhancement Ratio (SER) after 1 cycle of chemotherapy was an independent predictor of disease free survival. This finding could be of importance to use non-tumor SER values to risk stratify patients receiving neoadjuvant chemotherapy (see appendix).

Reportable Outcomes:

This work has resulted in 2 abstracts and 2 manuscripts (one submitted, one in preparation):
1. Rembert J., Klifa C., Lu Y., Gibbs J., Park C., Hylton N.: Breast Magnetic Resonance Imaging (MRI) enhancement beyond the tumor margin: Implications for radiation therapy, presented to San Antonio Breast Cancer Symposium, December 2005.

2. Rembert J., Hattangadi J., Klifa C., Gibbs J. , Lu Y. , Hwang J. , Park C., Hylton N.: "Breast Magnetic Resonance Imaging (MRI) enhancement beyond the tumor margin: Is there an association with treatment response?", presented at ASTRO, November 2006

3. Hattangadi J., Park C., Rembert J., Klifa C., Hwang J, Gibbs J., Hylton N.: Stromal Enhancement by MRI is associated with response to neoadjuvant chemotherapy", manuscript sent to the American Journal of Roentgenology (AJR) in July 2007, **see appendix**.

4. Klifa C., Lu Y., Gibbs J., Partridge S., Hylton N.:"Non-tumor breast tissue measures and response to neoadjuvant chemotherapy. Implications for outcome.", manuscript in preparation.

This work has also provided preliminary results for a NIH-R21 grant submission, involving identical measures as developed in this project, but using a combination of two modalities, MRI and Diffuse Optical Spectroscopy (DOS), to potentially define early measures of treatment response in patients undergoing pre-operative chemotherapy. This prospective study plans to recruit 30 patients from on-going clinical trials at our institution. This grant entitled "Breast Cancer Treatment Monitoring Tools Combining MRI and Optical Spectroscopy " was submitted on October 1st 2006, with Dr Klifa as Principal Investigator. Despite receiving a good score (143, 15%) that new project was not funded by NIH but by the California Breast Cancer Research Program (CBCRP) in May 2007.

Conclusions

In this study, we hypothesized that the imaging phenotype reflects tumor growth patterns that are influenced by the normal host tissue. We found that change in uninvolved breast tissue volume with treatment was correlated with tumor phenotypes and was shown to be predictive of recurrence. These results suggest that the host tissue may provide treatment response information. We are now studying a larger number of cancer patients to verify our findings as well as to define additional MRI quantitative measures that could also be correlated with tumor characteristics or treatment outcome.

Thanks to this retrospective study we have been focusing on improving the "host" tissue analysis by adding another modality (DOS) and therefore link treatment outcomes with structural, functional and physiological information on the breast tissue. We have received funding from the California Breast Cancer Research Program on May 2007 to start a project combining MRI and Diffuse Optical Spectroscopy (DOS) to study early treatment response in a prospective study involving women undergoing neoadjuvant therapy. We expect that combining multimodalities to analyze the host tissue may help detect early changes with treatment.

References:

1 Boetes, C., et al., *Breast tumors: comparative accuracy of MR imaging relative to mammography and US for demonstrating extent.* Radiology, 1995. **197**(3): p. 743-7.

2 Esserman, L., et al., *Utility of magnetic resonance imaging in the management of breast cancer: evidence for improved preoperative staging.* J Clin Oncol, 1999. **17**(1): p. 110-9.

3 Partridge, S.C., et al., *Accuracy of MR imaging for revealing residual breast cancer in patients who have undergone neoadjuvant chemotherapy.* AJR Am J Roentgenol, 2002. **179**(5): p. 1193-9.

4 Wasser, K., et al., *Evaluation of neoadjuvant chemotherapeutic response of breast cancer using dynamic MRI with high temporal resolution.* Eur Radiol, 2003. **13**(1): p. 80-7.

5 Esserman, L., et al., *MRI phenotype is associated with response to doxorubicin and cyclophosphamide neoadjuvant chemotherapy in stage III breast cancer*. Ann Surg Oncol, 8(6): p. 549-59, 2001.

6 Klifa C. et al, *Quantification of breast tissue index from MR data using fuzzy clustering*, IEEE Engineering in Medicine and Biology Society, Vol. 3, p.1667-70, 2004

Editorial Manager(tm) for American Journal of Roentgenology
Manuscript Draft

Manuscript Number:

Title: Stromal Enhancement by MRI is Associated with Response to Neoadjuvant Chemotherapy

Article Type: Original Research

Section/Category: Breast Imaging

Keywords: magnetic resonance imaging; microvascular density; breast stroma; neoadjuvant chemotherapy

Corresponding Author: Nola Hylton, PhD

Corresponding Author's Institution: University of California, San Francisco

First Author: Jona Hattangadi, MD

Order of Authors: Jona Hattangadi, MD; Catherine Park, MD; James Rembert, MD; Catherine Klifa, PhD; Jimmy Hwang, PhD; Jessica Gibbs; Nola Hylton, PhD

Abstract: Objective: Cancerous changes in breast tissue beyond the tumor margin have been shown to increase risk for recurrence. We hypothesize that signal enhancement ratios (SER) from dynamic contrast-enhanced breast MRI (DCE-MRI) can be used to analyze the contrast kinetics of microvasculature in breast stroma beyond the tumor margin.
Methods: SER analysis of non-tumor breast stroma was performed on DCE-MRI scans from 42 patients who received neoadjuvant chemotherapy for invasive breast cancer, both pre-treatment (Scan 1) and post one-cycle of chemotherapy (Scan 2). Stromal SER was characterized relative to clinical parameters, and univariate and multivariate analyses were performed to determine the association between stromal SER and disease-free survival (DFS).
Results: Median follow-up for the group was 52 months. Scan 1 mean stromal SER was significantly associated with change in both tumor size (p=0.04) and tumor volume (p=0.04) with chemotherapy. On univariate analysis, factors that were significantly associated with DFS included: pre-treatment tumor size (HR=0.33, p=0.012), pre-treatment tumor volume (HR=1.04, p=0.006), number of involved lymph nodes (HR=1.18, p=0.005) and Scan 2 mean stromal SER (HR=0.11, p=0.03). These factors were incorporated

into a multivariate model, which showed that only Scan 2 mean stromal SER was significantly associated with DFS (HR=0.11, $p<0.045$).

Conclusions: These findings indicate that breast stromal tissue, outside of the incident tumor, can be quantified using SER analysis on DCE-MRI. Stromal SER may be a potential indicator for overall outcome in breast cancer.

Suggested Reviewers:

Opposed Reviewers:

Title: Stromal Enhancement by MRI is Associated with Response to Neoadjuvant Chemotherapy

Authors: Jona Hattangadi MD[1], Catherine Park MD[2], James Rembert MD[2], Catherine Klifa PhD[1], Jimmy Hwang PhD[3], Jessica Gibbs[1], Nola Hylton PhD[1]

Addresses:

1. Magnetic Resonance Science Center
Department of Radiology
1 Irving Street, Room AC-109, Box 1290
University of California, San Francisco
San Francisco, CA 94143

2. Department of Radiation Oncology
1600 Divisadero St, MZ Bldg R H1031
University of California, San Francisco
San Francisco, CA 94143 - 1708

3. Comprehensive Cancer Center
Box 1297, 2340 Sutter A-740
University of California, San Francisco
San Francisco, CA. 94143 – 1297

Address of author who has moved:

James Rembert, MD
2001 Dwight Way
Berkeley, CA 94704

Corresponding Author:

Nola Hylton, PhD
Magnetic Resonance Science Center
Department of Radiology
1 Irving Street, Room AC-109, Box 1290
University of California, San Francisco
San Francisco, CA 94143
Phone: (415) 476-8856

Fax: (415) 476-8809
e-mail: Nola.Hylton@radiology.ucsf.edu

Acknowledgment of grants:

This work was supported by the Department of Defense (W81XWH-05-1-0518) and the National Institutes of Health RO1 (CA 069589).

ABSTRACT

Objective: Cancerous changes in normal appearing breast tissue beyond the tumor margin following lumpectomy and radiotherapy have been shown to increase risk for recurrence. However, the optimal treatment volume is not well defined, and modern imaging techniques have been optimized to characterize the primary tumor. We hypothesize that signal enhancement ratios (SER) from dynamic contrast-enhanced breast MRI (DCE-MRI) can be used to analyze the contrast kinetics of microvasculature in breast stroma beyond the tumor margin, which can be developed to improve local treatment.

Materials and Methods: SER analysis of non-tumor breast stroma was performed on DCE-MRI scans from 42 patients who received neoadjuvant chemotherapy for invasive breast cancer, both pre-treatment (Scan 1) and after one cycle of chemotherapy (Scan 2). Stromal SER was characterized relative to clinical parameters, and univariate and multivariate analyses were performed to determine the association between stromal SER and disease-free survival (DFS).

Results: Median follow-up for the group was 52 months. Scan 1 mean stromal SER was significantly associated with change in tumor size ($p=0.04$) and change in tumor volume ($p=0.04$) with chemotherapy. On univariate analysis, factors that were significantly associated ($p<0.05$) with DFS included: pre-treatment tumor size (HR=0.33, $p=0.012$), pre-treatment tumor volume (HR=1.04, $p=0.006$), number of involved axillary lymph nodes (HR=1.18, $p=0.005$) and mean stromal SER from Scan 2 (HR=0.11, $p=0.03$). These factors were then analyzed in a multivariate Cox proportional hazards model. The

only factor that was associated with DFS was Scan 2 mean stromal SER (HR=0.11, p< 0.045).

Conclusions: These findings indicate that breast stromal tissue, outside of the incident tumor, can be quantified using SER analysis on DCE-MRI. Stromal SER is a potential indicator for response to treatment and for overall outcome in breast cancer. However, these results should be validated in a prospective study.

Keywords: magnetic resonance imaging; microvascular density; breast stroma; neoadjuvant chemotherapy.

INTRODUCTION

Breast conserving therapy consisting of lumpectomy and radiation therapy (RT) is a commonly used approach for breast cancer treatment. Local recurrences occur despite modern imaging modalities and pathologic assessment. Invasive breast cancers have been shown to harbor abnormalities in adjacent tissue, outside of the primary tumor, indicating that the biology of the surrounding stroma may be a critical component to achieving optimal local control [1, 2]. In the past several years, tumor angiogenesis, which emanates in the stroma, has been shown to be necessary for tumor growth, and indeed, has been shown to begin with early pre-invasive lesions such as ductal carcinoma in situ [3].

Recent studies have shown in both human [4] and mouse [5] mammary carcinoma that tumor microenvironment affects tumor cells' angiogenic response. This growing body of research emphasizes the importance of understanding the role of normal stroma in breast cancer pathogenesis and the link to clinical outcome.

Magnetic resonance imaging (MRI) has had an evolving role in the management of breast cancer, with studies confirming the value of dynamic contrast-enhanced MRI (DCE-MRI) in cancer detection and diagnosis [6,7]. There is also substantial support in the literature that DCE-MRI has the ability to accurately predict the extent of residual disease in the breast after neoadjuvant chemotherapy [8,9]. The signal enhancement ratio (SER) is a calculated parameter that quantifies the kinetics of contrast enhancement on high spatial resolution DCE-MRI. Our group used DCE-MRI with SER analysis to correlate SER patterns with histopathology and microvessel density in breast tumors [10]. We found that areas with high SER values (early enhancement with rapid washout) are significantly correlated with high tumor vascularity. We hypothesized that SER analysis could be used as a non-invasive means of gaining clinically useful information about breast stroma outside the incident tumor in women with invasive breast cancer. This information could enhance our understanding of the local biology of disease and translate to improved local treatment and outcomes.

The purpose of this study was two-fold. First, we aimed to characterize the SER of radiographically normal fibroglandular stroma in patients with invasive breast cancer, before and after one cycle of neoadjuvant chemotherapy. Second, we tried to determine whether a correlation exists between stromal SER in the breast and clinical outcomes in patients with invasive breast cancer.

METHODS

Study Design

This study is a secondary data analysis of a prospective cohort of women with locally advanced breast cancer enrolled in an institutional neoadjuvant chemotherapy

breast cancer protocol at our institution between 1995 and 2002. An institutional review board approved the study protocol, and all subjects gave informed consent.

Patient Population

Between 1995 and 2002, 62 patients were enrolled in the neoadjuvant chemotherapy breast cancer protocol [11]. The patients all had stage II or III locally advanced invasive breast cancer, defined as tumors that have not spread beyond the breast and regional lymph nodes but may involve the skin of the breast or the chest wall. All patients had invasive breast cancer confirmed by pathology of core biopsy or fine needle aspiration specimens. Patients received four cycles of doxorubicin and cyclophosphamide (AC) chemotherapy that was given every three weeks. This was followed by 12 weekly cycles of taxane (T) in 12 patients. Patients were imaged with dynamic contrast-enhanced MRI (DCE-MRI) before treatment (Scan 1) and again after the first cycle of chemotherapy (Scan 2).

Four patients were excluded from our analysis for the following reasons: no pre-treatment MRI was obtained, inability to complete therapy, deviation from the therapeutic protocol, and loss to follow-up. An additional 16 patients were excluded for anatomic reasons that would prevent accurate SER measurement in radiographically normal breast stroma. These patients either had diffuse enhancement throughout the breast tissue on DCE-MRI, or were excluded for lack of sufficient fibroglandular stroma for evaluation. Sufficient fibroglandular stroma was defined as at least two centimeters of radiographically normal (non-enhancing) breast stroma extending from the edge of visibly enhancing tumor

The remaining 42 patients were included in this study. All patients underwent DCE-MRI before chemotherapy (Scan 1), and 33 patients after one cycle (Scan 2). Patient characteristics, clinical variables, and recurrence and outcome data were obtained from the pre-existing dataset.

Clinical Variables

Clinical variables were retrieved from the original cohort database. Patient age was recorded at the beginning of treatment. Pathologic data were determined from pathology reports. Recurrence and disease-free survival (DFS) was assessed for each patient based on clinical examination and mammographic imaging at 6-month or 1-year intervals after surgery. Length of DFS was defined as the time between the primary surgery (following neoadjuvant chemotherapy) and local or distant recurrence, or as the time to last follow-up in patients with no evidence of recurrence.

MRI Acquisition

As described in the original prospective study [11], MR imaging was performed on the involved (ipsilateral) breast only. Images were acquired on a 1.5-T Signa scanner (GE Healthcare) using a dedicated bilateral phased-array breast coil. A fat-suppressed 3D fast gradient-recalled echo sequence was used (TR/TE, 8/4.2; flip angle 20º; 2 repetitions.) [12]. The entire breast was covered with 60 slices, each 2-mm thick, acquired in the sagittal orientation. The contrast agent used was gadopentetate dimeglumine (Magnevist, Schering; Berlin, Germany), injected at a dose of 0.1 mmol/kg body weight, followed by a 10-ml saline flush. Three time points (t_0, t_1, t_2) were acquired during each MRI examination: a baseline scan before contrast agent injection (t_0),

followed by two sequential scans after contrast injection (t_1, t_2), yielding a temporal sampling of 0, 2.5, and 7.5 minutes.

SER Analysis

Three different patterns of signal increase and washout after contrast injection are shown in the red, green and blue curves in Figure 1(a). The blue curve shows a slow gradual increase in enhancement, more characteristic of normal tissue; the green curve shows early enhancement with little washout, essentially a plateau in signal intensity; the red curve shows a pattern of early enhancement with quick washout, which is more characteristic of highly vascularized tissue and neoangiogenic vessels.

These patterns are quantified with the signal enhancement ratio (SER), which compares enhancement in the first post-contrast image to enhancement in the second post-contrast image [13]. Figure 1(a) displays signal intensity (S) on the y-axis and time (t) on the x-axis. Images are acquired at three time points: t_0 or pre-contrast, t_1 at 2.5 minutes after contrast injection, and t_2 at 7.5 minutes after contrast injection. S_0, S_1, and S_2 represent the signal intensity at t_0, t_1, and t_2, respectively. SER is a unitless value defined as $(S_1-S_0) / (S_2-S_0)$, which is normalized and comparable from patient to patient and from scan to scan.

MRI Postprocessing and SER

In order to characterize normal-appearing breast fibroglandular tissue in the ipsilateral breast, regions of interest (ROI's) were created on the first post-contrast image (image acquired at t_1, 2.5 minutes after contrast injection), as shown in Figure 2. On a single representative sagittal slice containing visible enhancing tumor, five circular

regions of interest (ROI's), each 5mm in diameter, were placed extending radially from the tumor edge. The first ROI was placed within the visible tumor and the next four in normal appearing breast fibroglandular stroma. This is shown schematically in Figure 2: "T" inside the circle represents the tumor ROI, and "S" represents the stromal ROI's. A second set of similarly placed ROI's was obtained along a different radius of the same image if enough normal stroma was present. This process was repeated for Scan 2, and all attempts were made to place ROI's in approximately the same locations between Scan 1 (pre-treatment) and Scan 2 (post one-cycle chemotherapy).

Volumetric SER maps, which assign SER values on a voxel-by-voxel basis, were generated from contrast-enhanced fat-suppressed T1-weighted breast MR images using a customized software program developed at our institution [13]. The SER algorithm was initially designed to characterize breast tumors, and used a minimum threshold of at least 70% enhancement from baseline to 2.5 minutes after contrast injection to define malignancy. In this study, the aim was to assess breast fibroglandular stroma, a tissue compartment that enhances much less avidly, so the initial enhancement threshold was reduced to 20%. The ROI's created on the original t_1 images were superimposed on the SER maps to extract the mean SER within each ROI, excluding zero values. The ROI's were generated with the users blinded to the volumetric SER map, so as not to select areas of interest with higher SER values. Users were also blinded to the clinical outcome of each patient while conducting the MRI postprocessing and SER analysis.

Pre-test for Inter-Observer Variability

To assess the variability in ROI selection, two independent observers collected data as described above on MRI scans of 15 patients. The values obtained for stromal

SER were analyzed for any differences with respect to the user. The same two observers conducted the MRI postprocessing and SER acquisition for the entire cohort.

Statistical Analysis

Stromal SER values were evaluated as a function of distance from visible tumor and for effect of chemotherapy. Additionally, the associations between stromal SER and pathologic characteristics, clinical response, and disease recurrence were analyzed using the Wilcoxon and student's t-tests. Stromal SER values were dichotomized for the analyses, as described in the results section.

When analyzing DFS, patients without disease recurrence were censored. Univariate analyses using Kaplan-Meier log-rank tests and the Cox proportional hazards model were performed to identify variables associated with DFS. A hazard ratio (HR) and p-value were reported for each variable in comparison with length of DFS. Variables that were found to be significant in the univariate analysis were entered into multivariate Cox regression models, to identify significant predictors of DFS. DFS curves were produced using the Kaplan-Meier method. Statistical significance was established at $p<0.05$.

RESULTS

Inter-observer Variability

There was no significant difference in the mean SER values from ROI's measured between the two users on the same patient, or the entire sample population as a whole.

Patient Characteristics

Table 1 shows the characteristics of this cohort. Median age was 48.3 years (range 29.7 -71.5). Median follow-up was 52.1 months (range 17.6 – 86.7) in those patients who remained disease-free. In patients who recurred, median time to recurrence was 25.2 months (range 3.6 – 73.9). Mean pre-treatment tumor size, measured as the longest diameter of the tumor on MRI, was 4.69 cm. The majority of patients had invasive ductal carcinoma (83.3%) and many had grade 3 disease (52.4%) according to the Scarff-Bloom-Richardson grading system. More than half had positive axillary lymph nodes (57.1%) and this subset of patients had a mean of 4.38 positive nodes.

All but one patient received AC chemotherapy, and 10 of these patients received additional treatment with taxane. All patients underwent surgical resection following chemotherapy, with a fairly equal split between mastectomy and lumpectomy. Mean pathologic size, measured as longest diameter of lesion after surgical resection, was 2.93 cm. One patient had a complete pathologic response with neoadjuvant chemotherapy and showed no residual disease upon resection.

Disease-free Survival (DFS) and Recurrence

DFS of the entire study population, calculated using the Kaplan-Meier method, is displayed graphically in Figure 3. The 2-year DFS rate was 70% for the group of patients studied (n=42). Recurrences are shown in table form; there were 15 total recurrences (35.7% of patients), with 11 distant metastases and 4 local recurrences.

Tumor and Stromal SER Characterization

For each scan, SER values from each ROI in breast stroma were characterized relative to their position, essentially the distance from the edge of visible tumor. No relationship was found between the stromal SER of individual ROI's and their position relative to the tumor. As a result, all stromal ROI's were averaged to give a single mean stromal SER per scan for subsequent analyses. When we compared tumor with stromal SER values, as expected, the mean SER values from ROI's within visible tumor were significantly higher than stromal SER values ($p<0.0001$).

Mean tumor SER values decreased significantly with chemotherapy ($p=0.0002$). However, there was no significant change in mean stromal SER values with one cycle of chemotherapy (from Scan 1 to Scan 2).

Stromal SER and Recurrence

We attempted to assess the relationship between mean stromal SER values and recurrence. Both Scan 1 (pre-treatment) and Scan 2 (post one-cycle chemotherapy) mean stromal SER values were found to be significant predictors of tumor recurrence. Table 2 compares the mean stromal SER values of patients who did recur (n=15) versus those who did not (n=27). Patients who recurred had significantly lower mean stromal SER values at Scan 1 ($p=0.03$) and Scan 2 ($p=0.02$).

Mean stromal SER values from patients who recurred versus those who did not fell below and above 0.7, respectively. Thus, this value was used to dichotomize mean stromal SER as a variable for subsequent analyses.

Pre-treatment Stromal SER and Clinical Parameters

Scan 1 mean stromal SER values were associated with the nodal status of patients. Patients with positive axillary lymph nodes had lower Scan 1 mean stromal SER values ($p=0.02$). When this parameter was analyzed dichotomously, patients with Scan 1 stromal SER values of <0.7 had an average of 3.2 positive axillary nodes versus 1.1 positive nodes in those with values ≥ 0.7 ($p=0.03$).

Scan 1 mean stromal SER values was also associated with clinical response, as measured by change in tumor size (longest diameter of tumor, cm) and change in tumor volume (cc) on MRI from pre-treatment to completion of four cycles of neoadjuvant chemotherapy. Patients with Scan 1 mean stromal SER values of ≥ 0.7 had a greater decrease in tumor size ($p=0.04$) and tumor volume ($p=0.04$) with chemotherapy when compared with those patients with values of <0.7.

Univariate and Multivariate Analysis for DFS

Variables that were included in the univariate analysis for association with DFS were: age, pre-treatment tumor size, pre-treatment tumor volume, pathologic tumor size, nodal status, number of positive axillary lymph nodes, Scarff-Bloom-Richardson grade, surgery type, chemotherapy, recurrence type, Scan 1 mean stromal SER and Scan 2 mean stromal SER, Table 1. Stromal SER was analyzed dichotomously (<0.7, ≥ 0.7).

Those variables that were significantly associated with the length of DFS in the univariate analysis are shown in bold in Table 1. Both tumor size ($p=0.012$) and tumor volume ($p=0.006$) before treatment were significantly associated with DFS. Number of positive lymph nodes was also significantly correlated with DFS in this analysis. Scan 2 mean stromal SER, analyzed as a dichotomous variable, was significantly associated with

DFS (p=0.03). With a hazard ratio of 0.11, a higher value of Scan 2 mean stromal SER is associated with a decreased risk of recurrence.

The four factors found to be significant in the univariate analysis were incorporated into the multivariate Cox proportional hazards model. Only Scan 2 mean stromal SER (HR 0.11, $p<0.045$) remained independently associated with DFS.

Subset Analysis of Scan 2 Stromal SER and DFS

Figure 5 displays graphically the Kaplan-Meier curves for length of DFS, with patients divided based on Scan 2 mean stromal SER values <0.7 or ≥ 0.7. There is a significant difference in DFS in the two groups (p=0.012, log-rank test), with a three-year DFS rate of 82.4% in the group with stromal SER ≥ 0.7 and 47.9% in the group with values <0.7.

DISCUSSION

This study is based on the idea that the radiologically normal appearing, non-neoplastic stroma surrounding tumor in the breast may play a crucial role in tumor pathogenesis and response to treatment. Microvessel density within tumors is known to correlate with poor prognosis of breast cancer patients [14,15], but little is known about the nature of extratumoral microvessel density and its influence on clinical outcome. Predicting outcome in the treatment of breast cancer is difficult, as the disease itself is biologically and clinically heterogeneous. Imaging is non-invasive and can be performed frequently, making it a powerful tool to guide future therapy. We have previously shown that DCE-MRI parameters including the volume and initial diameter of the tumor can predict response to neoadjuvant chemotherapy in patients with breast cancer [11]. In the

present study, we used SER analysis of DCE-MRI to study radiographically normal appearing fibroglandular tissue in breast cancer patients receiving neoadjuvant chemotherapy. We found that higher mean SER values in breast stroma after one cycle of chemotherapy are significantly correlated with a better outcome—fewer recurrences and longer DFS.

We found no significant relationships between the SER of individual stromal ROI's and their position relative to the tumor. Thus, stromal ROI's were averaged to give a mean stromal SER value per scan, representing the average values of the extratumoral tissue extending 2 cm away from the tumor (4 stromal ROI's x 0.5 cm each). SER values measured in the extratumoral stroma were always <1, indicative of normal vasculature with gradual increase in enhancement, whereas SER values within visible enhancing tumor were >1, characteristic of neoangiogenic tissue. SER values within the tumor decreased significantly with one cycle of chemotherapy, as we would expect from previous studies [12]. However, stromal SER did not change significantly with chemotherapy.

Stromal SER was predictive of clinical outcome even at time of diagnosis. Both pre-treatment (Scan 1) and post one-cycle (Scan 2) stromal SER were significant predictors of tumor recurrence, as shown in Table 1. This indicates not only that changes induced by chemotherapy may be predictive, but that the underlying baseline physiological characteristics of the breast stroma are important factors. Interestingly, patients who recurred had significantly lower values (<0.7) of mean stromal SER on both Scan 1 and Scan 2. Scan 1 mean stromal SER values were also associated with clinical parameters. Patients with lower values were more likely to be node-positive, and those

with values <0.7 had more positive nodes and a smaller decrease in tumor size with four cycles of chemotherapy. These findings may be related: those patients with lower mean stromal SER values at Scan 1 had more lymph node involvement, less clinical response to treatment, and were more likely to recur.

The univariate analysis showed that pre-treatment tumor size and tumor volume (both obtained from Scan 1 MRI) were significant predictors of DFS, as shown in Table 1. This strong correlation validates the use of DCE-MRI in the neoadjuvant setting to help predict clinical outcome. Scan 2 mean stromal SER was also a significant predictor of length of DFS, with values ≥ 0.7 conferring a significantly decreased risk of recurrence (HR 0.11, p=0.03). Number of positive lymph nodes was the only other prognostic factor associated with DFS in the univariate analysis. This may be due to the relative homogeneity and small numbers of this group with regard to overall risk factors.

In the multivariate model for DFS, Scan 2 mean stromal SER was found to be a significant independent predictor of DFS ($p<0.045$), associated with a dramatically reduced risk of recurrence (HR 0.11) in patients with values ≥ 0.7. Figure 4 shows graphically the significant difference in DFS based on dichotomized Scan 2 mean stromal SER (p=0.012). This finding indicates that changes associated with one cycle of chemotherapy may further predict outcome in patients receiving neoadjuvant treatment.

Our findings showed an inverse correlation of stromal SER and recurrence in this neoadjuvant population. One hypothesis to explain these results is that a higher microvessel density in the extratumoral region might ensure better delivery of the chemotherapeutic agent to the tumor. This would result in a better clinical response and decreased likelihood of recurrence after surgery. It has been reported that the

effectiveness of molecular medicines to treat cancer may be jeopardized if they cannot efficiently penetrate tumor tissue [16]. However, histopathologic correlation between stromal SER levels and markers of vascularity in stromal tissue are necessary to validate the biological meaning of stromal SER.

There are a few limitations to this study. The study population is relatively young in age with large, high grade tumors—most of them invasive ductal carcinoma. Because of the high number of recurrences in this cohort, we were able to study the association of imaging findings with clinical outcome. However, the distinct nature of this cohort limits our ability to generalize these findings to populations of patients with better prognoses, i.e. those that were detected earlier. Also, the automated algorithm that measures SER was optimized for use in tumor, where there is avid enhancement. While we lowered the peak enhancement threshold in our methodology, there might be other adjustments that could be made to best analyze the contrast kinetics of stromal tissue. Further studies of stromal SER are ongoing. While the results of this study are promising, they should be validated in a larger, prospective study.

ACKNOWLEDGEMENTS

This work was supported by the Department of Defense (W81XWH-05-1-0518), the National Institutes of Health RO1 (CA 069589), and the Doris Duke Charitable Foundation.

REFERENCES

1. Deng G, Lu Y, Zlotnikov G, Thor AD, Smith HS. Loss of heterozygosity in normal tissue adjacent to breast carcinomas. *Science* **1996;** 274:2057-2059

2. Li Z, Moore DH, Meng ZH, Ljung BM, Gray JW, Dairkee SH. Increased risk of local recurrence is associated with allelic loss in normal lobules of breast cancer patients. *Cancer Res* **2002;** 62:1000-1003

3. Guidi AJ, Schnitt SJ, Fischer L, et al. Vascular permeability factor (vascular endothelial growth factor) expression and angiogenesis in patients with ductal carcinoma in situ of the breast. *Cancer* **1997;** 80:1945-1953

4. Edel MJ, Harvey JM, Papadimitriou JM. Comparison of vascularity and angiogenesis in primary invasive mammary carcinomas and in their respective axillary lymph node metastases. *Clin Exp Metastasis* **2000;** 18:695-702

5. Monsky WL, Mouta Carreira C, Tsuzuki Y, Gohongi T, Fukumura D, Jain RK. Role of host microenvironment in angiogenesis and microvascular functions in human breast cancer xenografts: mammary fat pad versus cranial tumors. *Clin Cancer Res* **2002;** 8:1008-1013

6. Hylton N. Magnetic resonance imaging of the breast: opportunities to improve breast cancer management. *J Clin Oncol* **2005;** 23:1678-1684

7. Esserman L, Wolverton D, Hylton N. Magnetic resonance imaging for primary breast cancer management: current role and new applications. *Endocr Relat Cancer* **2002;** 9:141-153

8. Abraham DC, Jones RC, Jones SE, et al. Evaluation of neoadjuvant chemotherapeutic response of locally advanced breast cancer by magnetic resonance imaging. *Cancer* **1996;** 78:91-100

9. Gilles R, Guinebretiere JM, Toussaint C, et al. Locally advanced breast cancer: contrast-enhanced subtraction MR imaging of response to preoperative chemotherapy. *Radiology* **1994;** 191:633-638

10. Esserman L, Hylton N, George T, Weidner N. Contrast-Enhanced Magnetic Resonance Imaging to Assess Tumor Histopathology and Angiogenesis in Breast Carcinoma. *Breast J* **1999;** 5:13-21

11. Partridge SC, Gibbs JE, Lu Y, et al. MRI measurements of breast tumor volume predict response to neoadjuvant chemotherapy and recurrence-free survival. *AJR Am J Roentgenol* **2005;** 184:1774-1781

12. Partridge SC, Gibbs JE, Lu Y, Esserman LJ, Sudilovsky D, Hylton NM. Accuracy of MR imaging for revealing residual breast cancer in patients who have undergone neoadjuvant chemotherapy. *AJR Am J Roentgenol* **2002;** 179:1193-1199

13. Partridge SC, Heumann EJ, Hylton NM. Semi-automated analysis for MRI of breast tumors. *Stud Health Technol Inform* **1999;** 62:259-260

14. Uzzan B, Nicolas P, Cucherat M, Perret GY. Microvessel density as a prognostic factor in women with breast cancer: a systematic review of the literature and meta-analysis. *Cancer Res* **2004;** 64:2941-2955

15. Gasparini G. Prognostic value of vascular endothelial growth factor in breast cancer. *Oncologist* **2000;** 5 Suppl 1:37-44

16. Minchinton AI, Tannock IF. Drug penetration in solid tumours. *Nat Rev Cancer* **2006;** 6:583-592

Table 1: Patient Characteristics Associated with DFS [#]

Characteristic	Value*	HR[↕]	p-value
Age (years): < 50 ≥ 50	26 (62) 16 (38)	0.707	0.56
Pre-treatment tumor size, mean (range), cm	4.69 (1.1-10.8)	**1.332**	**0.012**
Pre-treatment tumor volume mean (range), cc	18.9 (0.1-71.3)	**1.037**	**< 0.01**
Pathologic tumor size at resection mean (range), cm	2.93 (0-11.5)	1.144	0.14
Nodal status Positive Negative	 24 (57.1) 18 (42.9)	1.832	0.31
# of positive nodes, mean	4.38	**1.176**	**< 0.01**
Scharff Bloom Richardson Grade 1 2 3	 6 (14.3) 13 (31.0) 22 (52.4)	1.167	0.661
Surgery type Lumpectomy Mastectomy	 22 (52.4) 20 (47.6)	1.143	0.80
Chemotherapy		1.212	0.78

AC	30 (71.4)		
AC/T	11 (26.2)		
FEC	1 (2.4)		
Recurrence			
Local	4 (9.5)	3.37	0.1
Distant	11 (26.2)		
Scan 1 Mean Stromal SER			
< 0.7	28 (66.7)	0.719	0.614
≥ 0.7	14 (33.3)		
Scan 2 Mean Stromal SER		**0.108**	**0.037**
< 0.7	19 (57.6)		
≥ 0.7	14 (42.4)		

[#] Significant characteristics are shown in bold.

* Values are expressed as number of patients (percentage) unless otherwise indicated.

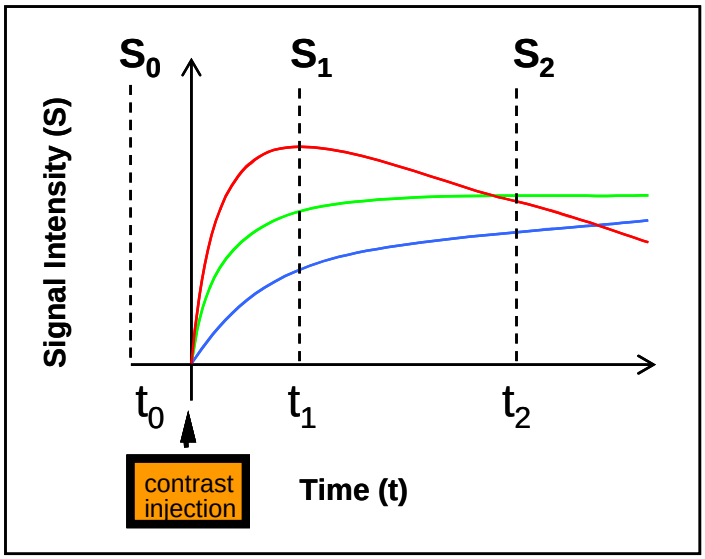
[↕] Hazard ratios for continuous variables represent the increased risk imparted by a unit increase in the variable.

Table 2: Mean Stromal SER and Recurrence

		+ Recurrence	- Recurrence	p-value
Mean Stromal SER (95% CI)	Scan 1	0.632 (0.583-0.681)	0.728 (0.667-0.790)	p=0.03
	Scan 2	0.632 (0.523-0.742)	0.769 (0.704-0.834)	p=0.02

FIGURES

a.



b.

Signal enhancement ratio (SER):

$$SER = \frac{(S_1 - S_0)}{(S_2 - S_0)}$$

Fig. 1—Analysis of Contrast Kinetics with SER (signal enhancement ratio)

a.) Graph of signal intensity (S) versus time (t). S_0, S_1, and S_2 represent the signal intensity of images obtained at t_0 (pre-contrast injection), t_1 (2.5 minutes after contrast injection), and t_2 (7.5 minutes after contrast injection), respectively. The three curves in red, green, and blue display the different patterns of signal increase and washout that are seen with time.

b.) Signal enhancement ratio (SER) is the increase in signal intensity at t_1 relative to baseline (t_0), divided by the increase from baseline to t_2.

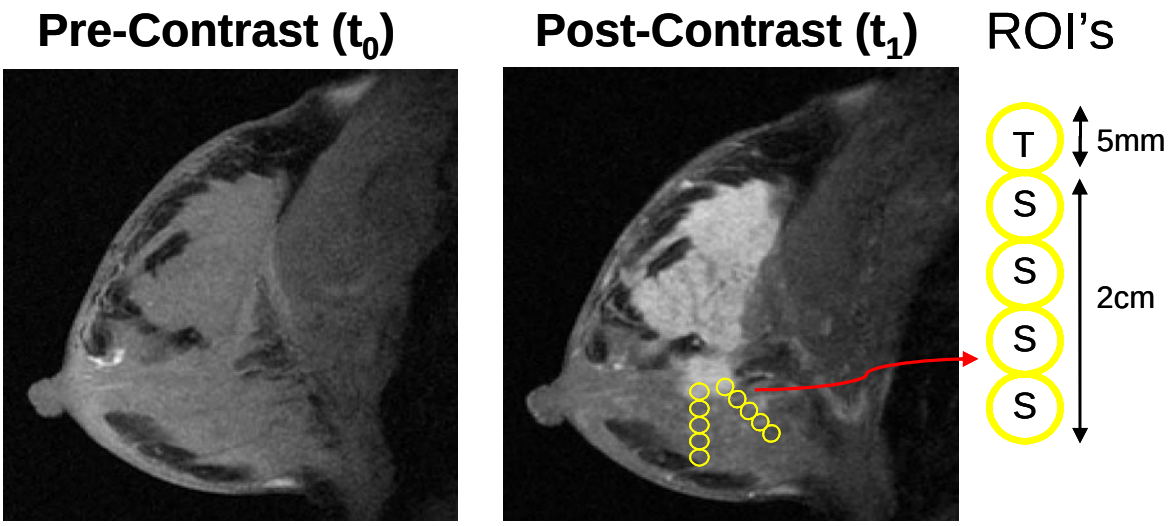


Fig. 2— ROI selection on DCE-MRI. Five regions of interest (ROI's), each 5mm in diameter, were created on the first post-contrast image (at t_1) extending radially from the tumor edge. The first ROI was placed within visible tumor (T) and the next four in normal appearing breast fibroglandular stroma (S). A second set of similarly placed ROI's was obtained along a different radius for each scan, if enough normal (non-enhancing) stroma was present.

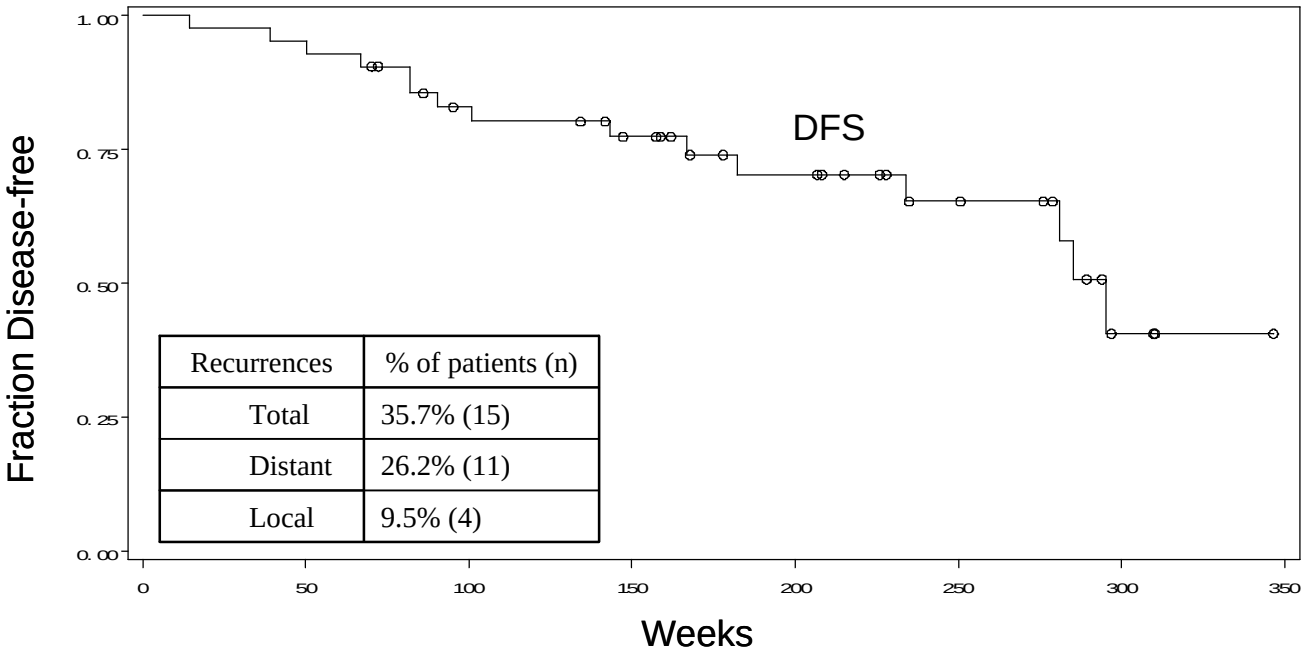


Fig. 3—Disease free survival (DFS) and recurrence among study population. DFS distribution curve of entire patient cohort (n=42) is displayed graphically. Recurrences are shown: 15 total (11 distant, 4 local).

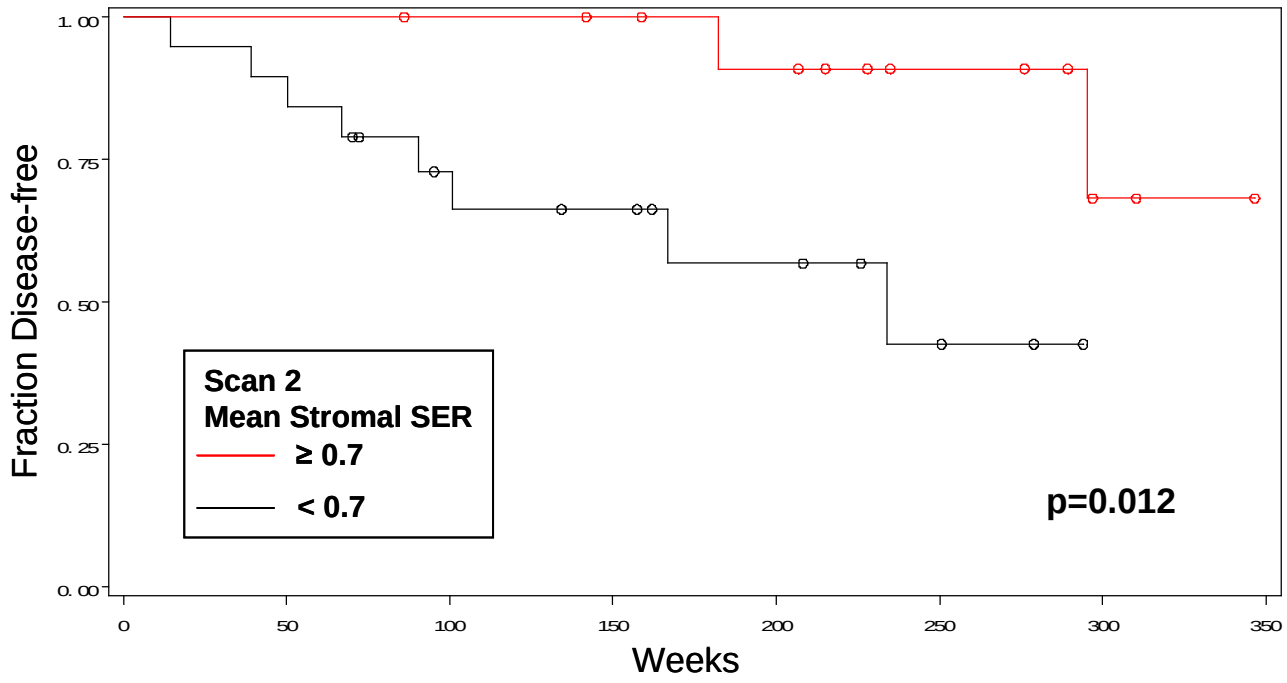


Fig. 4—Association between Scan 2 Mean Stromal SER and disease free survival (DFS). Significantly longer DFS was observed in those patients with Scan 2 mean stromal SER values ≥ 0.7 (p=0.012, log-rank test).